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Treatment of Severe Laryngeal Papillomatosis With Intralesional Injections of Cidofovir [(*S*)-1-(3-Hydroxy-2-Phosphonylmethoxypropyl) Cytosine]

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Respiratory papillomatosis is a rare and often severe disease, usually localized in the larynx. It may cause respiratory distress and even life-threatening obstruction of the airways. Treatment is generally based on the evaporation of the lesions with a CO_2 laser, but microsurgery, cytotoxic and/or cytostatic drugs, interferons, and vaccines are also used. Cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] (HPMPC) was shown to suppress the growth of tumors induced by rabbit papillomavirus as well as human papillomavirus (HPV). The efficacy of cidofovir was assessed in 17 patients with severe respiratory papillomatosis. Cidofovir at a concentration of 2.5 mg/ml was injected directly in the different laryngeal papillomatous lesions during microlaryngoscopy under general anesthesia. Biopsies were taken before the treatment was started both for anatomopathology and viral typing. HPMPC kinetics in serum was monitored in three patients, the drug levels being determined by high-performance liquid chromatography. Complete disappearance of the papillomatosis was observed in 14 patients. Four patients relapsed and were successfully treated again with cidofovir. Of the three remaining patients, one progressed while under treatment with cidofovir, after an initial marked response. One patient had a partial remission and remained stable for more than 1 year after the last injection. He had a very aggressive and extensive disease originally. Finally, one patient was lost to follow-up after four injections. Intratumoral injections of cidofovir for the treatment of severe laryngeal papillomatosis is a powerful new therapeutic approach for this disease. Treatment was well tolerated, and no significant side effects were noted. ***J. Med. Virol. 54:219–225, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: cidofovir; papillomavirus; larynx

INTRODUCTION

Human papillomavirus (HPV) is a species-specific DNA virus responsible for a broad range of diseases that are characterized by epithelial cell proliferation. With recent advances in molecular biology, more than 70 different types of HPV have been identified and classified, based on target tissues, carcinogenicity, and nucleic acid homology of the genome. HPV is responsible for warts, benign self-limiting proliferative lesions that may regress spontaneously after a period of time. Some HPV types are also associated with naturally occurring cancers. In humans, the presence of the virus has been demonstrated in a rare dermatological disorder, epidermodysplasia verruciformis, but it is in genital cervix dysplasia and neoplasia that the role of HPV has been studied most frequently [Schneider and Koutsky, 1992].

Recurrent respiratory papillomatosis, mostly caused by HPV type 6 or 11, or, sometimes, by type 16 or 18, is a rare and severe disease with an unpredictable course: Some tumors grow very slowly while others have a fulminant course [Kashima et al., 1993]. The most frequent localization is the larynx (90%), but the lesions can spread along the entire respiratory and upper digestive tracts. It may lead to respiratory distress and even life-threatening progressive obstruction of the airways. The papillomatous lesions tend to recur, and regular intervention, occasionally as frequently as every 2 weeks, may be required for years. Dissemination

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to the lungs may ensue. Respiratory papillomatosis is a disease that mostly starts in early childhood, but may occur at any age [Kashima et al., 1992; Doyle et al., 1994a]. Many therapeutic approaches have been developed for recurrent laryngeal papillomatosis. To date, three approaches have been used: chemical inhibition of the papilloma growth (antimetabolites, hormones, podophyllum), physical removal by a CO_2 laser, which has become the method of choice, and enhancement of the immune response (vaccines and interferons) [Phelps and Alexander, 1995]. Cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, HPMPC, Vistide®] has been recognized as a potent inhibitor of the replication of herpesviruses (HSV-1, HSV-2, VZV, CMV, EBV, HHV-6), adenoviruses, and papillomaviruses [Kurtzman et al., 1993; Snoeck et al., 1995; Van Cutsem et al., 1995; Hitchcock et al., 1996]. Recently, it was demonstrated that cidofovir interferes with the growth of tumors induced by Shope papillomavirus in the cottontail rabbit [Kurtzman et al., 1993]. Local applications of cidofovir gel were found to effect a complete regression of severe anogenital papillomatosis in immunocompromised patients [Snoeck et al., 1995]. Similarly, it was shown that cidofovir when injected directly into a severe esophageal papillomatous tumor is able to achieve a complete remission (now persisting for more than 4 years) [Van Cutsem et al., 1995].

The results of a study are described on the treatment of patients with severe papillomatosis of the respiratory tract by intralesional administration of cidofovir under general anaesthesia.

PATIENTS AND METHODS

All the patients included in this study between July 1994 and March 1997 had severe laryngeal papillomatosis, of a refractory nature in some patients. Most of the patients were adults, reflecting the population of the Laryngology Department of the Hospital. The lesions were all glottic except in two patients. The diagnosis was confirmed in all cases by a biopsy. All patients signed an informed consent. Blood was screened for hematological and chemical parameters (particularly from a renal function viewpoint because of the known nephrotoxic potential of cidofovir) before inclusion in the study, and also during the treatment period. A total of 17 patients, seven females and ten males, were included in the study. Their age ranged from 11 to 77 years with a mean of 44 years (median 49 years). The previous treatment of the patients is summarized in Table I. Patients 7, 12, and 15 had no previous treatment.

TABLE I. Previous Treatment

Treatment	No. of patients
Laser alone	6
Microsurgery	1
Laser and microsurgery	4
Multiple treatments	3
Laser + microsurgery + interferon + interleukin (IL-2)	1
Laser + microsurgery + interferon	2
No previous treatment	3

Cidofovir was provided by Gilead Sciences (Foster City, CA) as a solution for intravenous use. Further dilutions were made to reach the final concentration of 2.5 mg/ml. Vials were kept at room temperature. Cidofovir is stable for several months at room temperature. Patients were put under total intravenous anaesthesia (TIVA), using the JET ventilation technique. A direct laryngoscopy was carried out, and cidofovir was injected via a microsurgical needle under microscopic control directly in each lesion of the larynx. The patients were admitted to hospital the day before the injection and discharged the day of the injection or the day thereafter. In the beginning of the therapeutic cycle, the injections were given every other week until a clear clinical response was noted. Then, the frequency of the injections was reduced. When the last macroscopic lesion had disappeared, the patient received two additional sets of injections at the previous site of the lesions. Follow-up of the patients thereafter was on an outpatient basis by indirect laryngoscopy every 4 weeks. A biopsy was taken before the first injection for histopathology. HPV typing was performed in some cases. HPV DNA detection was carried out on biopsy tissue using polymerase chain reaction (PCR) [Tachezy et al., 1994]. Briefly, consensus primers MY11 and MY09 amplified a 450–base pair fragment in the HPV-L1 region [Manos et al., 1989]. A control primer set (PCC04 and GH20), which amplified a 286–base pair beta-globin gene fragment, was included to confirm the presence of an adequate amount of amplifiable DNA. About 10 μl of the reaction product was digested with *Rsa*I, and the resulting fragments were resolved on a horizontal 3% agarose gel, yielding a restriction fragment pattern specific for HPV-6 and/or HPV-11. For confirmation, and also to resolve ambiguous banding patterns, PCR products were subcloned in a pGEM-T vector (Promega, Madison, WI), and sequenced on a Pharmacia ALF automated sequencer.

To determine the systemic distribution of cidofovir after intralesional administration, serum dosages were undertaken in two patients, 10 ml blood being taken from a vein of the arm at different times after the end of injection, for up to 48 hr. The levels of cidofovir in the samples were determined by high-performance liquid chromatography (HPLC) [Wachsman et al., 1996]. Briefly, serum samples were inactivated and deproteinized by treatment with ice-cold methanol during 30 min at 4°C, followed by centrifugation. The supernatants were vacuum-evaporated, and, after reconstitution in elution buffer, the extracts were analyzed by ion-pairing reverse-phase HPLC, with a 0.40 × 125-mm LiChrospher 60 RP-select B column (particle size 5 μm) purchased from Merck (Darmstadt, Germany). The HPLC system was from Waters (Milford, MA) and was equipped with a photodiode-array detector. Elution

TABLE II. Cidofovir Treatment Regimens for the Different Patients Subject of This Study

Patient			Number of injections	Mean amount of cidofovir (2.5 mg/ml) injected (ml)	Min-Max (ml)	Duration of treatment (months)	Duration of remission after end of treatment (months)
Sex		Age (years)					
1[a]	M	49	9	6.6	3–10	8	17
2	F	11	9	7.2	4–9	5	(27)[b]
3	F	14	7	7.6	6–9	4	11
4	M	30	13	6.4	2–10	8	18
5	M	51	15	6.9	2–12	9	10
6	F	52	3	4.3	3–6	2	27
7	F	23	4	6	3–9	2	16
8[a]	F	44	5	6.8	4–10	2.5	10
			4	6.8	6–9	2	2
			3	6.5	5–8	3	6
9	M	32	11	8.3	3–17	13	18[c]
10	M	74	3	4	3	2	20
11[a]	F	57	7	5.5	3–6	3	10
12	M	62	2	3.5	1–6	1	15
13[a]	F	16	3	4	2–6	1.5	5
			1	5	5	—	7
14	M	66	3	8	6–9	1	11
15	M	66	9	6.4	2–9	6.5	3
16	M	22	4	7.4	5–10	2	(—)[d]
17	M	77	7	4.6	2–6	4.5	2

[a]Patients presenting with relapse.
[b]Patient under combined therapy (cidofovir, alpha-interferon, and laser) after progression under cidofovir alone.
[c]Partial responder, stabilized after completion of the treatment.
[d]Lost to follow-up.

was carried out with a 25-min gradient system from 100% buffer (5 mM tetra-n-butylammonium hydrogen sulphate; 5 mM ammonium dihydrogen phosphate; pH 7.5) to 25% acetonitrile. The retention time of cidofovir was ~8 min. Identification of cidofovir in the samples was based on peak retention time and spectral analysis. The concentration of cidofovir in the samples was determined from the $UV_{275\ nm}$ absorbance and was calculated from a calibration curve that was obtained by the analysis of standards prepared from blank patient material. The lower limit of cidofovir detection in serum was 0.2 μg/ml.

RESULTS

A total of 121 cidofovir injections were undertaken in the 17 patients evaluated in this study. The modalities of the cidofovir injection for the different patients are summarized in Table II. The number of injections per patient varied from three to 15. The mean volume of cidofovir solution injected per patient per session was 4 to 8 ml, while the duration of total treatment varied from 1 month to 13 months. The minimum amount injected during a session was 1 ml and the maximum was 17 ml (Table II).

Patients 3, 4, 6, 7, 10, 12, 14, 15, and 17 received treatment until the last macroscopic lesion had disappeared. The mean duration of treatment for these patients was 3.3 months (range: 1–8 months), with a mean duration of complete remission of 13.6 months (range: 2–27 months). All of these patients are presently disease free (Fig. 1).

Patient 5 had a good initial response, but a persisting lesion at the level of the right arythenoid necessitated multiple injections resulting in a slow reduction in the size of the lesion. Finally, the last lesion at the level of the right arythenoid disappeared after a total of 15 injections over 9 months. A biopsy taken after the last injection was negative. This patient has now been disease free for 10 months (Fig. 2).

Patient 8 received the first treatment regimen consisting of five injections, which led to a complete disappearance of the broadly disseminated lesions. A biopsy taken 1.5 months after the last injection gave a doubtful picture not fully compatible with papillomatosis. After 10 months' remission she presented with recurring lesions located in the same area. A second treatment regimen was then started immediately, and after two sessions more than 90% of the lesions had disappeared. This patient received a third and fourth injection, the latter when macroscopic lesions were no longer observed. A second relapse was diagnosed after 2 months of follow-up during which the patient was disease free. Two months later the patient underwent a new series of three injections leading again to complete macroscopic disappearance of the lesions. No signs of relapse have been noted 6 months after the last injection.

Patient 1 had a complete response after 8 months of treatment, with improvement of his voice. He relapsed 17 months after the last injection. He is now under a new cycle of intralesional injections with cidofovir. Similarly, patient 11 is under a new treatment cycle, since she relapsed 5 months after the last injection of a 3-month treatment period. Patient 13 was cured after 3 injections of cidofovir and relapsed 6 months later. She received one more injection and is disease free after a

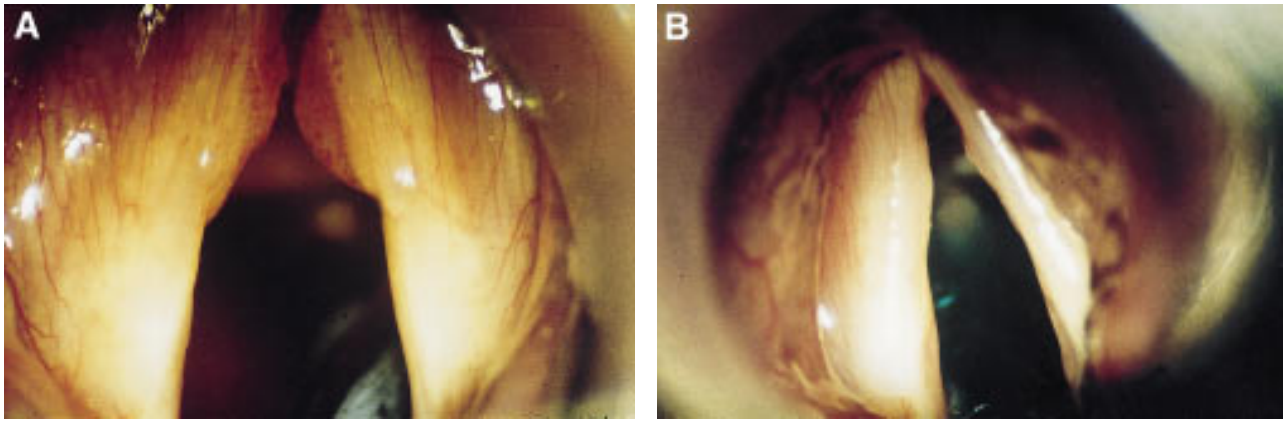

Fig. 1. Patient 7 vocal cords before **(A)** and after **(B)** treatment.

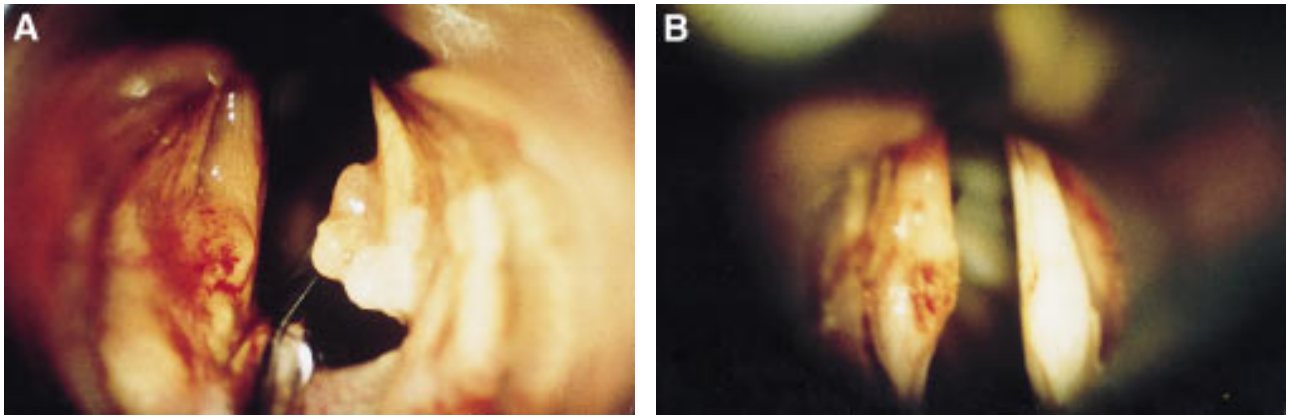

Fig. 1. Patient 5 vocal cords before **(A)** and after **(B)** treatment.

7-month follow-up period. The relapses of patients 1 and 8 were multifocal, whereas patients 11 and 13 had localized relapses.

Patient 9 had a very severe case of multitreated laryngeal papillomatosis mostly located on the vocal cords. Laser therapy was necessary as frequently as every other week. This patient received 11 injections over a 13-month period with gradually longer intervals between the injections. This patient could not be injected until the lesions had disappeared completely. Nevertheless, at 18 months after the last injection, the growth rate of the lesions was reduced drastically and his voice had improved. During the 18 months following the last injection, the patient did not need any further intervention, his voice analysis parameters being stable.

Finally, patient 2, a girl of 11 years, had had severe relapsing laryngeal papillomatosis for 8 years. She had already received a multitude of different therapies with laser therapy, microsurgery, and interferon (Table I). After a marked initial response, the lesions reappeared notwithstanding cidofovir therapy. Then laser therapy combined with systemic interferon alpha (Roferon®) was begun. Later on, cidofovir was installed again, combined with laser therapy and interferon alpha therapy. This patient's lesions have now been stable under this treatment given every 3rd week.

Two of the patients (2 and 3) developed an immediate cutaneous rash after cidofovir injection on one occasion. Nevertheless, such a rash is also known to happen to patients under TIVA. Rashes were not further observed during the following sessions of treatment of these two patients. Patient 5 experienced a headache after each injection, which responded to symptomatic treatment. Patient 10 received three injections of cidofovir. He reported precordialgia on the days following the injections. This patient was known for coronary insufficiency, but his electrocardiogram (ECG) did not show any changes.

Patient 16 had a severe disease disseminated at the subglottic level. Only the glottic level was treated with cidofovir, the remaining lesions being healed with laser therapy. There was a clear response at the level of the injected lesions. The patient was lost to follow-up for the study due to progression of the lesions at the subglottic level.

The biopsies taken from all the patients prior to treatment were characteristic of papillomatous lesions. In addition, patients 15 and 17 showed lesions microscopically compatible with verrucous carcinoma. After

completion of the treatment, late biopsies taken from patient 15 were characterized by persistent hyperkeratosis, but in situ hybridization for HPV was negative.

HPV typing, by PCR, of the initial biopsies was performed for two patients and indicated the presence of HPV type 6 and/or 11.

Five series of cidofovir serum dosages were performed in three different patients. For patient 2, 10 min after the intralesional injection of cidofovir, the drug concentration in serum was 0.36 μg/ml. In patient 9, serum dosages were done at three different occasions. Cidofovir could be detected at one occasion, the serum concentration of cidofovir being 0.59, 0.60, and 0.42 μg/ml at 5, 10, and 15 min, respectively, after the end of the injection. For patient 1 and the two other series of serum dosages, the passage of cidofovir in the bloodstream, from the intralesional injections, could not be demonstrated.

None of the patients had changes in blood chemistry. Hematological measurements were stable. Mucosal fibrosis or necrosis were not observed at the site of the injections.

DISCUSSION

Recurrent respiratory papillomatosis is a disease characterized by the presence of lesions mostly at the laryngeal level which can lead to respiratory distress and airway obstruction [Kashima et al., 1993]. The first symptom is usually a change in voice, i.e., hoarseness. An aggressive form occurs mostly in children under 5 years of age [Chipps et al., 1990; Kattner and Clark, 1993; Simma et al., 1993; Doyle et al., 1994a]. This form is characterized by a higher rate of subglottic involvement. Epidemiologically, it appears that the mode of transmission of the disease is different between the adult and the juvenile forms of recurrent respiratory papillomatosis [Kashima et al., 1992], while for the disease and its evolution the distinction is more arbritary [Hartley et al., 1994]. The mean recurrence interval varies from years to as frequently as 2 weeks. In children, spontaneous resolution with puberty has been reported, but this is still controversial [Doyle et al., 1994a].

The etiologic agent is the human papillomavirus (HPV) [Phelps and Alexander, 1995]. HPV types 6 and 11 are associated with respiratory tract papillomatosis [Duggan et al., 1990; Hartley et al., 1994], and specific subtypes may be correlated with disease severity [Mounts et al., 1982]. An association has been demonstrated between maternal cervical HPV infection and the incidence of recurrent respiratory papillomatosis in children [Gissmann et al., 1982; Doyle et al., 1994a]. Retrospectively, the presence of maternal condylomata acuminata has been reported in a high percentage of children with respiratory papillomatosis. Transmission typically follows vaginal delivery but has also been documented after caesarian section. The child with respiratory papillomatosis typically presents between 2 and 3 years of age [Fletcher, 1991; Kashima et al., 1992; Shah et al., 1996].

In adults, the source of the viral contamination is not as clear as for children. Oral sexual practice could be a source [Kashima et al., 1992]. Medical staff may be at risk as the viral particles recovered in the vapor of the CO_2 laser used for the treatment of patients with HPV lesions could transmit the disease. It is also possible that in adults, laryngeal papillomatosis represents reactivation of a quiescent virus acquired at birth. Using polymerase chain reaction (PCR), it has been demonstrated that HPV is present in normal tissue of patients known to have respiratory papillomas. The potential reservoir site of reinfection is more commonly located in the lower respiratory tract [Smith et al., 1993]. The suggestion of an intralaryngeal reservoir is supported by the finding that the infecting HPV type in recurrent disease within an individual patient remains unchanged, suggesting that reinfection is endogenous. The trigger(s) for reactivation are unknown.

Recurrent respiratory papillomatosis is usually a benign tumor of the larynx. If the disease spreads, it may invade the trachea, the bronchi (2–17%), and the bronchioli and alveoli (<1%) [Gaylis and Hayden, 1991; Williams et al., 1994]. In the case of respiratory distress, a tracheotomy may be necessary, although this may lead to spreading of the lesions to the tracheotomy as well as to the midthoracic trachea, bronchi, and lungs. If a tracheotomy cannot be prevented, the canula should be removed as soon as possible. There are some preferential sites for recurrent respiratory papillomatosis that include the nasal vestibuli, the nasopharyngeal surface of the soft palate, the midzone of the laryngeal surface of the epiglottis, the upper and lower margins of the ventricle, the undersurface of the vocal folds, the carina, and the bronchial spurs. All these sites have a common histologic feature: they are at the junction between squamous and ciliary epithelium. These sites thus appear to be at risk for papillomatous formation [Kashima et al., 1993]. Only rarely does the papilloma become invasive by undergoing malignant degeneration. It has been shown that patients with higher degrees of cellular atypia are more likely to have more recurrences and are at a higher risk of malignancy. These degenerations have been described in both laryngotracheal and bronchoalveolar areas, and the reported incidence is 2–3%. Most of the cases of malignant degeneration that have been reported, occur in patients who have received radiotherapy [Chipps et al., 1990; Gaylis and Hayden, 1991; Wilde et al., 1994]. In a recent case report, early biopsies in the course of the disease demonstrated HPV types 6 and 11. As the disease evolved into carcinoma, types 6 and 11 remained present, and, in addition, type 16 became increasingly prevalent [Doyle et al., 1994b].

Treatment of respiratory papillomatosis at present is usually based on CO_2 laser vaporization, but microsurgical resection with mechanical devices is still being used. Also cryosurgery, cauterization, ultrasound, topical chemotherapy, steroids, podophyllin, tetracycline, autogenous vaccine, interferon, and immune stimulators have been described. CO_2 laser surgery has re-

mained the preferred technique for the treatment of laryngeal papillomatosis. With the improvement of the technique and the selection of the appropriate CO_2 laser emission parameters, there is a definite decrease in the soft-tissue complications [Chipps et al., 1990; Kattner and Clark, 1993; Phelps and Alexander, 1995]. Several studies have evaluated interferon in animal models [Gangemi et al., 1994] as well as in clinical trials [Leventhal et al., 1991] with good results. Mostly, the effects of interferons on human papillomavirus infections were studied in patients with the anogenital forms [Cirelli and Tyring, 1994; Phelps and Alexander, 1995]. More recently, several antivirals have been tested in patients with laryngeal papillomatosis [Phelps and Alexander, 1995]. Preliminary studies undertaken with ribavirin in the cottontail rabbit papillomavirus model showed a reduced number of warts, later onset of warts, and a reduced tumor mass [Ostrow et al., 1991]. Ribavirin has also been administered to patients in a preliminary study combining the drug and surgery. The results observed were encouraging [McGlennen et al., 1993]. A few reports have mentioned controversial results with acyclovir (Zovirax®) in the treatment of recurrent respiratory papillomatosis. The number of patients was small, and therefore no definitive conclusions could be drawn [Endres et al., 1994; Kiroglu et al., 1994]. In fact, the mechanism of antiviral action of acyclovir, which is essentially based on phosphorylation by the virus-induced thymidine kinase, does not support its potential activity against HPV [Phelps and Alexander, 1995]. Of the acyclic nucleoside phosphonate derivatives, 9-(2-phosphonomethoxyethyl)guanine was the first shown to be a potent inhibitor of the growth of tumors induced by the papillomavirus in the cottontail rabbit model [Kreider et al., 1990]. Later, cidofovir was shown to be one of the most potent inhibitors in the same model [Kurtzman et al., 1993]. Recently, the potential of cidofovir to inhibit HPV replication was demonstrated in a papillomatous hypopharyngeal/esophageal tumor induced by HPV type 16/18 [Van Cutsem et al., 1995] and in three AIDS patients with genital papillomatosis [Snoeck et al., 1995].

The results of our study open new perspectives for the treatment of severe and relapsing laryngeal papillomatosis. There is a clear effect of cidofovir on the progression of these papillomatous tumors. Of the 17 patients treated, 14 showed a complete remission including one patient (patient 5) with a prolonged treatment for a persisting lesion of the arythenoid. Among them, one patient (patient 8) had two relapses but is now in complete remission at 6 months after the third treatment episode. Patients 1, 11, and 13 each had an episode of relapse after a follow-up of 13, 5, and 6 months, respectively. Patient 9 has now had stabilized lesions for 18 months after his last injection, whereas before starting cidofovir therapy, he needed laser surgery as frequently as every other week. Only one patient (patient 2) had a recurrence while under cidofovir therapy, after an initial marked response to the treatment.

We consider these preliminary results as highly encouraging. More studies and a longer follow-up are needed to draw definitive conclusions, particularly with regard to the recurrence rate. Local injections of cidofovir were free of any local or systemic toxicity. Since HPV does not encode for a specific viral DNA polymerase, and the compound seems to affect specifically those cells that contain HPV, a particular mechanism of action different from that of cidofovir against herpesviruses is postulated. The mode of action of cidofovir against HPV-induced cell proliferation is under investigation.

No inflammation, scarring, or fibrosis have been observed at any of the sites where cidofovir was injected. In addition, cidofovir was always used at concentrations far below the systemic toxic concentration. Very low concentrations of cidofovir were detected in the blood of two of the three patients in whom pharmacokinetics were determined, excluding any risk of systemic and, particularly, renal toxicity.

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